

Various Methods to Detect Microalbumin in Early Detection of Nephropathy

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ABSTRACT

Article Info

Volume 8, Issue 4

Page Number : 274-279

Publication Issue

July-August-2021

Article History

Accepted : 08 July 2021

Published : 15 July 2021

Background : Microalbuminuria (defined as urinary albumin excretion of 30-300 mg/day, or 20-200 µg/min) is an earlier sign of vascular damage. It is a marker of general vascular dysfunction and nowadays is considered a predictor of worse outcomes for both kidney and heart patients. Microalbuminuria could be taken also, as an indicator of insulin resistance and of the increased renal and cardiovascular risk associated with metabolic syndrome. Renal involvement is a pivotal development in diabetes and microalbuminuria is generally the first clinical sign of renal dysfunction in diabetics. It is demonstrated that cardiovascular and renal risk is elevated even in the high normal range of microalbuminuria (below 30 mg/day). **Material and method;** The cross sectional study was conducted in department of biochemistry and medicine in Guru Gobind Singh Medical College and Hospital, Faridkot. 50 patients, who suffering with diabetes and hypertensive were enrolled for study. **Result;** Out of 50 diabetes and hypertensive patients, 31 males and 19 females. The p values are not significant so all the 3 methods can be used for detecting microalbumin in diabetic and hypertensive patients. In our study we found that microalbumin by antigen antibody method as provided by point of care instrument (ICHROMA) gives sensitivity of 88% and specificity of 80.1%. Albumin creatinine ratio by laboratory method is cheap and simple method with sensitivity of 86.0% but specificity of 42.8% **Conclusion:** Albumin creatinine ratio by dye based strip method is a good marker to detect microalbumin in early detection of nephropathy in diabetic and hypertensive individuals. Microalbuminuria by antigen antibody reaction detection by ichroma provides good sensitivity and specificity. On the other hand ACR by lab method cannot substitute ACR by strip due to its low specificity.

Keywords : Microalbumin, Diabetics Nephropathy, Albuminuria, Hypertensive, Ichroma.

I. INTRODUCTION

The kidneys are two bean-shaped organs found on the left and right sides of the body in vertebrates. They are located at the back of the abdominal cavity in the retroperitoneal space. In adults they are about 11 centimetres (4.3 in) in length. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder. The nephron is the structural and functional unit of the kidney. Each adult kidney contains around one million nephrons. The nephron utilizes four processes to alter the blood plasma which flows to it: filtration, reabsorption, secretion, and excretion. Via one or more of these mechanisms, the kidney participates in the control of the volume of various body fluid compartments, fluid osmolality, acid-base balance, various electrolyte concentrations, and removal of toxins. Filtration occurs in the glomerulus: one-fifth of the blood volume that enters the kidneys is filtered.^[1]

Constituting 60% of the total plasma protein, albumin contributes to about 80% of the colloid osmotic pressure, and binds a variety of ligands such as fatty acids, bilirubin, calcium, hormones, and certain drugs. Albumin is excreted in the urine in very small amounts of less than 30 mg per day in healthy individuals. It is assumed that albumin passes through the glomerular filtration barrier, and is reabsorbed by the proximal tubular cells by receptors such as megalin and cubulin, after which it is delivered to the lysosomal system and broken down to amino acids which are delivered back into the circulation.^[2,3] Increased amounts of albumin can appear in the urine, resulting from increased glomerular filtration and/or altered tubular reabsorption. Albuminuria as an established risk marker! The excretion of small amounts of albumin in the urine (microalbuminuria,

30-300 mg/24 hour) has been established as one of the earliest indicators of nephropathy in patients with diabetes mellitus.^[4,5,6,7,8]

Different methods for the detection of microalbumin: HPLC, Immunonephelometry, Dip stick Method, Radioimmunoassay, Fluorescence immunoassay method. These all are high end methods its not possible for small and medium laboratories to perform these tests due to the high instrument cost and high test cost. In recent trend specific/dedicated instruments are used or commonly A:C Ratio is used. We can measure microalbumin by antigen antibody reaction and with laboratory methods too.

II. METHODS AND MATERIAL

The study was conducted in Department of Biochemistry in collaboration with the Department of Medicine in Guru Gobind Singh Medical College Faridkot. Ethical clearance was taken from institutional ethical committee. Proper informed consent was taken from all the Participants. 50 patients, who were suffering with diabetes and hypertensive attending the OPD of Department of medicine, was taken. They was subjected to detailed history and examination, biochemical and special testing according to the pretested perfoma.

Sample collection : 5 ml venous blood was drawn from each subject under aseptic conditions for routine and special investigations and Spot urine sample is collected. The blood sample is transferred in plain vacutainer and will be used for investigation. After the formation of blood clot, centrifugation was done at 3000 r.p.m for 10 minutes to separate serum. This serum was assessed for the various investigations. Serum biochemistry was performed on fully automated chemistry analyzer based on

Spectrophotometric method. Parameters that were determined include Plasma glucose, Renal function tests (urea, creatinine).

MICROALBUMINURIA WAS DETECTED BY 3 METHODS

- a) ACR by dye based strip method.
- b) Based on antigen-antibody reaction Read on ICHROMA (POCT).
- c) A:C Ratio by laboratory method.

III. RESULTS AND OBSERVATION

The study was carried out in the Department of Biochemistry in collaboration with Department of Medicine, Guru Gobind Singh Medical College, Faridkot with an objective to detect microalbuminuria in the early detection of nephropathy. An attempt was also made to compare various methods for the detection of microalbuminuria in the early detection of nephropathy. For this purpose, a total 50 patients with the history of Diabetes & Hypertensive were enrolled for study.

Table 1 : Showing Age Distribution of Patients

Age(Years)	Male	Female	Total(N)	Percentage
18-30	02	04	06	12.0
31-40	07	03	10	20.0
41-50	09	07	16	32.0
51-60	08	04	12	24.0
61-70	05	01	06	12.0
Total	31	19	50	100.0

Table 1 showing the age distribution of patients with mean age of 45.34 ± 12.93 years and range 18-70 years. Maximum numbers (32%) of patients were from the age group of 41-50 years followed by (24%) in the age group of 51-60 years.

Table 2 : Shows the Sensitivity and Specificity of Microalbuminuria by Ichroma and A:C Ratio by laboratory method in comparison to ACR by Strip method.

	Microalbuminuria by Ichroma	A:C Ratio by lab Method
Sensitivity	88.6%	86.0%
Specificity	80.1%	42.8%

Table 2 shows the Sensitivity and Specificity of various instruments. The sensitivity and specificity of ichroma is 88.6%, 80.1%. and A:C Ratio by lab method is 86.0%, 42.8% indicating that ACR by lab methods is sensitive but specificity is low.

Table 3 : Mean value of ACR by Strip, microalbuminuria by Ichroma and A:C Ratio by lab method in Diabetic Patients.

Diabetic					
ACR by Strip (mg/dl) Mean±S.D		Microalbuminuria By Ichroma (mg/l) Mean±S.D		A : C Ratio by lab method (mg/dl) Mean±S.D	
Male	Female	Male	Female	Male	Female
104.66 ± 101.19	86.91 ± 47.79	99.00 ± 103.83	79.06 ± 53.05	185.04 ± 261.27	107.05 ± 51.32

Table 4 : Mean value of ACR by Strip, microalbuminuria by Ichroma and A:C Ratio by lab method in Hypertensive Patients

Hypertensive					
ACR by Strip (mg/dl) Mean±S.D		Microalbuminuria By Ichroma (mg/l) Mean±S.D		A : C Ratio by lab method (mg/dl) Mean±S.D	
Male	Female	Male	Female	Male	Female
132.46 ± 101.89	133.51 ± 95.27	131.85 ± 107.88	125.91 ± 93.83	363.89 ± 642.00	175.51 ± 146.54

The table 4 shows ACR by Strip values in males and females in hypertensive patients was deranged with mean value of (132.46 ± 101.89) mg/dl and (133.51 ± 95.27) mg/dl. Microalbuminuria by Ichroma in males and females in hypertensive patients was deranged with mean value of (131.85 ± 107.88) mg/l and (125.91 ± 93.83) mg/l. And A : C Ratio values in males and females in hypertensive patients was deranged with mean value of (363.89 ± 642.00) mg/dl and (175.51 ± 146.54) mg/dl. (p>0.05).

IV. DISCUSSION

The Kidneys are small, dark organs that lie against the dorsal wall in a retroperitoneal position.[9] They receive some protection from the lower part of the rib cage.[10] An adult kidney is about 12 cm long, 6 cm wide, and 3 cm thick.[11]. The mean age of study group (as shown in table 1) showed the maximum number (32%) of patients were from the age group of 41-50 years followed by (24%) in the age group of 51-60 years. Mean age of Patient was 45.34 ± 12.93 years. Out of 50 patients included in the present study, there were 31 males and 19 females giving a ratio of 1.6:1 in the study group. Random Blood glucose was

deranged with mean value of 174.36 ± 56.14 mg/dl in Study Group. Urea has mean value of 32.76 ± 12.52 mg/dl. Creatinine has mean value of 0.81 ± 0.23 mg/dl. Random Blood glucose was deranged with mean value of 202.33 ± 38.59 mg/dl in Diabetic patients and Random Blood glucose was in range with mean value of 118.40 ± 25.98 in Hypertensive patients.

ACR by Strip was deranged with mean value of 91.58 ± 69.45 mg/g and Microalbumin by Ichroma was deranged with mean value of 85.66 ± 72.19 mg/l in patients and A:C Ratio by laboratory method was deranged with mean value of 208.12 ± 349.62 mg/dl. Values above the normal range are early markers of

nephropathy. The % of patients in various categories of CKD by various methods. ACR by Strip, microalbumin by Ichroma, A:C Ratio by method A1 Category (08,10,04)%. A2 Category (82,78,78)%. A3 Category (10,10,18)%. The categories of albuminuria are: A1 (ACR <30mg/g) Normal to mildly increased. A2 (ACR 30-300 mg/g) Moderately increased. A3 (ACR >300mg/g) Severely increased. the sensitivity and specificity of microalbumin by Ichroma is (88.6%, 80.1%). The sensitivity and specificity of A:C Ratio by lab method is (86.0%, 42.8). This shows that ACR by lab method is not useful in identifying true negative cases.

Mean value of Albuminuria patients by various methods with values between (<30 mg/g). ACR by Strip (18.45 ± 04.08) mg/dl, microalbumin by Ichroma (08.73 ± 03.17) mg/l, A:C Ratio by lab method (21.95 ± 07.98) mg/dl. The mean value of Microalbuminuria patients by various methods with values between (30 - 300 mg/g). ACR by Strip (106.31 ± 68.10) mg/dl, Microalbumin by Ichroma (93.16 ± 71.29) mg/l, A:C Ratio by lab method (98.66 ± 47.43) mg/dl. The mean value of Macroalbuminuria patients by various methods with values above (300 mg/g). ACR by strip (-), microalbumin by Ichroma (-) and A:C Ratio by lab method (706.77 ± 629.53) mg/dl. the percentage of patients with diabetic nephropathy is 54.76% and patients with hypertensive nephropathy is 45.23%. ACR by Strip values in males and females in diabetic patients was deranged with mean value of (104.66 ± 101.19) mg/dl and (86.91 ± 47.79) mg/dl and microalbuminuria by Ichroma in males and females in diabetic patients was deranged with mean value of (99.00 ± 103.83) mg/l and (79.06 ± 53.05) mg/l. and A:C Ratio by laboratory method values in males and females in diabetic patients was deranged with mean value of (185.04 ± 261.27) mg/dl and (107.05 ± 51.32) mg/dl.(p>0.05).

ACR by Strip values in males and females in hypertensive patients was deranged with mean value

of (132.46 ± 101.89) mg/dl and (133.51 ± 95.27) mg/dl. Microalbuminuria by Ichroma in males and females in hypertensive patients was deranged with mean value of (131.85 ± 107.88) mg/l and (125.91 ± 93.83) mg/l. And A:C Ratio values in males and females in hypertensive patients was deranged with mean value of (363.89 ± 642.00) mg/dl and (175.51 ± 146.54) mg/dl. (p>0.05).

The p values are not significant so all the 3 methods can be used for detecting microalbumin in diabetic and hypertensive patients. In our study we found that microalbumin by antigen antibody method as provided by point of care instrument (ICHROMA) gives sensitivity of 88% and specificity of 80.1%. Albumin creatinine ratio by laboratory method is cheap and simple method with sensitivity of 86.0% but specificity of 42.8%.

V. CONCLUSION

Albumin creatinine ratio by dye based strip method is a good marker to detect microalbumin in early detection of nephropathy in diabetic and hypertensive individuals. Microalbuminuria by antigen antibody reaction detection by ichroma provides good sensitivity and specificity. On the other hand ACR by lab method cannot substitute ACR by strip due to its low specificity.

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ABBREVIATIONS : ACR = Albumin creatinine ratio
HPLC = High performance liquid chromatography
OPD = Out Patient Department

Cite this article as :

Jagroop Singh, Dr. Manpreet Kaur, "Various Methods to Detect Microalbumin in Early Detection of Nephropathy", *International Journal of Scientific Research in Science and Technology (IJSRST)*, Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 8 Issue 4, pp. 274-279, July-August 2021. Available at doi : <https://doi.org/10.32628/IJSRST21837>
Journal URL : <https://ijsrst.com/IJSRST21837>